A FURTHER NOTE ON THE OCCUR-RENCE OF ANCYLOSTOMES RESEMBLING *NECATOR AMERICANUS* AMONGST DOMESTIC PIGS IN AMAZONAS

BY

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(Received for publication, April 25, 1923.)

In a previous note (1922) the author gave a brief description of Necators obtained from domestic pigs in Amazonas and reached the conclusion that, although of smaller size, the worm was indistinguishable from the human parasite *Necator americanus*. About the same time Ackert and Payne (1922) described a hookworm from the gut of the domestic pig in Trinidad. They stated that although this worm resembled *Necator americanus*, it exhibited certain differences which in their opinion were of specific value and accordingly they gave it the name *Necator suillus*. They elaborated their arguments in a later paper (1923). The points on which they base their conclusion that the pig worms differ from the human are the following :—

(I) 'The new species is somewhat smaller.'

(2) 'The buccal capsule much smaller proportionately.'

(3) 'As a rule the dorsal turn in the neck is not so pronounced as in *Necator americanus*.'

(4) 'In N. suillus the lateral lancets are broadly wedge-shaped in profile, while those of the large Necator are cusp-shaped. The ventral lancet is slender in side view pointing towards the base of the dorsal tooth in N. suillus while this lancet in the larger species is broader and points approximately towards the tip of the dorsal tooth.'

(5) 'The dorsal rays in the new species are shorter, while their

terminal branches are actually longer than these structures in the larger N. americanus.'

(6) 'In comparing specimens of these two species, cleared in glycerine, one is struck by the large so-called body cavity in the females of N, americanus, as contrasted with a much smaller one in N. suillus.'

(7) 'Concerning the males of these two species, the most striking differences are the proportions and shape of the bursa when closed. In N. *americanus* this organ is about as long as wide, and is distinctly funnel-shaped, the distal edges being flared like the bell of a trumpet, while the bursa of N. *suillus* is distinctly longer than wide and is more cup-shaped.'

(8) 'A conspicuous difference between these two species seen under higher magnification is the form of the head papillae. In *N. suillus* each lateral papilla unites with the corresponding dorso-lateral one, enclosing a conspicuous cam-shaped depression, a condition not true of *N. americanus*. Further it may be noted that in *N. americanus* the distal ends of the dorsal, lateral and ventral papillae are more or less beaded or constricted, while in *N. suillus* no such structures occur at the ends of the papillae.'

(9) 'Another rather constant difference is the shape of the externo dorsal ray. In N. suillus this ray, which is of nearly equal width throughout its length, makes a sharp lateral turn near its distal end; while in N. americanus the width of this ray is variable and the turn at the tip is less pronounced.'

(10) 'Finally the spicules show constant differences. The average length of the spicules of N. *americanus* is double that of the spicules of N. *suillus*. In the latter species both shafts terminate distally in the membranelle as recurved hooks, while in N. *americanus* only one shaft ends as a recurved hook, the other terminating in a nearly straight line.'

In view of the work of Ackert and Payne the writer has re-examined the pig ancylostomes from Amazonas and compared them with ancylostomes obtained from the human host in Amazonas and Jamaica, with special attention to the points mentioned above.

(I) Length of the two worms. Table I shows that, whereas the average size of the pig ancylostome is distinctly smaller, yet its maximum length is equal to the minimum length of the human parasite from Jamaica, and greater than that of the human parasite from Amazonas. The length cannot therefore in itself be used as a distinguishing character.

TABLE I.

Showing	the	lengths	of	Necators	obtained	from	pig	and	human	hosts.
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			Ma	ales		Females				
		Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres	Number measured	Maximum length in millimetres	Minimum length in millimetres	Average length in millimetres	
pig, Amazonas	••••	28	6.2	4*5	5*1	64	8.2	5*5	6.5	
o human host, Jamaica		28	9.0	6*5	7.8	64	13.0	8.5	10.0	
o human host, Amazonas	••••	28	8.0	5.0	6•8	64	11.2	7°5	ð. 1	

(2) Size of buccal capsule. It appears from Table II that the buccal capsule is proportionately greater in the pig than in the human ancylostome; this is the reverse of Ackert and Payne's findings. Much reliance cannot, however, be placed on small differences in these measurements, as well-marked variations in shape from the normal oval of the

TABLE II.

Showing the measurements of the buccal capsules in Necators obtained from pig and human hosts.

	Males							Females						
	NT 1	Max	imum	Mini	mum	Ave	rage	Number	Max	imum	Mini	mum	Ave	rage
	Number measured	Dorso- ventral diam.	Lateral diam.	Dorso- ventral diam.	Lateral diam.	Dorso- ventral diam.	Lateral diam.	measured	Dorso- ventral diam.	Lateral diam.	Dorso- ventral diam.	Lateral diam.	Dorso- ventral diam.	Lateral diam.
1, Amazonas	14	μ 66	μ 59	μ 59	μ 51	μ 60	$\mu_{5^{\circ}}$	31	μ 81	μ 74	μ 66	μ 59	μ 68	μ 57
lman host,	15	74	57	47	44	59	50	15	85	68	57	54	70	61
l nan host, a nas	15	64	61	51	44	61	46	15	85	68	64	47	74	60

mouth capsule were frequently encountered; another difficulty is that any variations in the angle from which the head is viewed will give rise to different results in the measurement of the mouth capsule.

(3) Dorsal curvature of anterior part of body. No such constant differences as those described by Ackert and Payne were observed in the anterior curvature.

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(4) Ventral lancets, lateral lancets and dorsal tooth. An examination was made of a large number of worms from both pig and man, but no constant differences in the lancets or dorsal tooth were found; the dorsal tooth and the ventral and lateral lancets of both worms showed great variation in size, shape, and angle of projection, as is illustrated in fig. I,



FIG. 1. The three projections in each drawing from above downwards are V.l.=Ventral lancet; L.l.=Lateral lancet; D.t.=Dorsal tooth. A, B, C, D, E and F=Necator americanus from human host, Amazonas; G, H, I, \mathcal{J}, K and L=Necators from domestic pig, Amazonas.

which represents camera lucida outlines drawn from unselected material. With regard to the angle of projection of the ventral lancets it is of interest to note that Ackert and Payne in their last paper depict these lancets as projecting at almost precisely the same angle in both N. americanus and N. suillus.

(5) Length of the dorsal rays. The results of measuring the dorsal rays in eighteen worms from pigs and in twenty-four from human material are shown in Tables III and IV.

TABLE III.

Showing measurements of dorsal rays and their branches in Necators obtained from pig and human hosts.

	Length in	microns of	dorsal ray	Len of inner	gth in micro branch of d	ons orsal ray	Length in microns of outer branch of dorsal ray		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
rig, Amazonas	95'0	65.0	80°0	34°0	18.0	26°4	16.0	10*4	13.9
a ica	130.0	97'0	111.2	36*4	26*0	29'9	19'0	11.2	14°3
r uman host, Ir zonas	120'0	78.5	110.2	32°5	19.2	25*9	15.0	7*8	11.8

TABLE IV.

owing ratio of length of dorsal ray and its branches to total length of the worm in Necators obtained from pig and human hosts.

	R to tot	atio of lengt of dorsal ray al length of	h worm	R of inner to tot	atio of lengt branch of d al length of	h orsal ray worm	Ratio of length of outer branch of dorsal ray to total length of worm		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
on ig, Amazonas	I: 57	1:92	1:71	1:183	I: 333	1:215	I : 343	I : 528	I:402
on uman host,	1:59	1:80	1:69	I : 2 06	I: 307	1:257	I: 394	і : 726	I:538
an uman host, Ar conas	1:51	I:89	1 : 65	I : 224	I: 357	I: 277	I : 414	1:1023	I : 610

From Table IV it follows that the average of the ratios of the lengths of the branches of the dorsal ray to that of the worm is slightly greater in the case of the pig Necators than in the human, but the maximum and minimum values overlap to such an extent as to make this point of no specific value. From Table III it can be seen that Ackert and Payne's statement that the actual lengths of the branches of the dorsal ray are greater in *N. suillus*, does not hold good for the Amazonas material.

(6) Size of the body cavity. Eight worms from the pig contrasted

with eight N. americanus from the human host in Brazil, showed no proportionate difference in the size of the body cavity.

(7) The shape and proportions of the bursae in the two worms. The dimensions of the closed bursa from its ventral aspect, of six Necators from the pig were 0.26 mm. long by 0.24 mm. broad, while those of six Necators from the human host in Brazil were 0.38 mm. long by 0.41 mm. broad. It is difficult to say when a bursa is completely closed and various stages between cup and funnel shape were observed both amongst Necators from the human and from pig hosts, but these variations in shape appear to depend entirely on the degree of approximation of the two halves of the bursa.

(8) The head papillae. The union of the lateral and dorso-lateral papillae as described by Ackert and Payne was clearly visible in the majority of the pig specimens, in others, however, no such union could be traced; it was, moreover, also present in many of the Necators of man, though possibly not as often as in those of the pig. The beading on the papillae was also found to be a variable factor and was seen at times in both worms.

(10) Length and shape of the spicules. The results of measuring the spicules of twelve worms from pigs in Amazonas and of those obtained from the human host in the same locality are recorded in Table V.

TABLE V.

Measurements of the spicules of Necators obtained from pig and human hosts in Amazonas.

		Length o	f worm in m	illimetres	Length of	spicules in 1	Ratio of length of spicul to total length of worn			
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Ave
From pig, Amazonas	••••	6.0	4*5	5*3	0.62	0*38	°*47	I:8°4	1:13.9	1:
From human host, Amazonas	••••	8.7	6.2	7°3	0*98	0°82	0,01	I : 7°I	1:10.6	I : 5

It appears from Table V that the average length of the spicules in the worms from man are nearly double the length of those in the worms from the pig. The average of the ratios of the length of the spicules to that of the body are, however, respectively $\mathbf{I}: \mathbf{8}$ and $\mathbf{I}: \mathbf{II}$, and it must moreover be noted that the maximum ratio in the case of the pig ($\mathbf{I}: \mathbf{8}\cdot \mathbf{4}$) is greater than the minimum ratio in the case of man ($\mathbf{I}: \mathbf{I0}\cdot \mathbf{6}$), and consequently this can hardly be regarded as a reliable point of distinction. Ackert and Payne's statement that both spicules in the pig Necator terminate in the membranelle as recurved hooks in contrast to N. *americanus* in which only one spicule is hooked, was not found to be constantly true of the worms from Amazonas, one hooked and one nearly straight spicule being very common amongst the pig Necators,



FIG. 2. *A*, *B*, *C* and D = Spicules of Necators from domestic pig, Amazonas. *E*, *F*, *G* and H = Spicules of *Necator americanus* from human host, Amazonas.

while in the case of the Necators from man both spicules frequently showed well-marked hooks. These points are illustrated in fig. 2.

Size of ova. Ackert and Payne give the average size of the ova of the pig Necators from Trinidad as 63μ by 37μ , the ova of the pig Necators from Amazonas were found to measure on an average 64μ by 39μ .

ATTEMPTS TO INFECT PIGS WITH NECATOR AMERICANUS

Ackert and Payne (1923) performed several experiments to ascertain whether N. *americanus* from the human host can mature in pigs; they also carried out experiments in pigs with cultures of N. *suillus*. For the sake of brevity, the results of Ackert and Payne's experiments are condensed in Table VI.

TABLE VI.

Summarising the results of Ackert and Payne's attempts to infect domestic pigs with larvae of N. americanus obtained from human sources.

No. of pig	No: of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks	
I	2841 2700 3000	16.6.21 20.6.21 27.6.21	} ' Infective '	Not stated	10.8.21	3 adult N. suillus	Faeces free from Nemate ova or larvae at time experiment	
2	2841	16.6.21	' Infective '	Not stated	10.8.21 8 adult N. suillus		Faeces free from Nemate ova or larvae at time experiment	
3	1850 12000 4000	27.9.21 29.9.21 4.10.21	} ' Infective '	By mouth on bread	3.12.21	7 adult N. suillus	In spite of oil of Cher podium treatment, p 3, 4, and 5 all 1 Ancylostome ova at ti of experiment	
4	7500 9900	29.9.21 1.10.21	Not stated Sheathed	Placed on shaven skin	3.12.21	6 mature N. suillus		
5	Nil			_	3.12.21	3 adult N. suillus	Used as a control pigs 3 and 4	
6	5000		' Infective''	Not stated	73 days after infection	No hook- worms found	Owing to the difficulty obtaining pigs free fr ancylostomes in Trinid this experiment was co ducted in Manhatt Kansas	

Ackert and Payne claim to have shown from these experiments that it is not possible to infect pigs with *Necator americanus*, either by the mouth or by the skin. This claim appears to the writer to rest entirely on their ability to distinguish between *Necator americanus* and *Necator suillus*, for in every experiment, except in the case of Pig 6, where the method of administering the larvae is not stated, *N. suillus* was found at the autopsy. It is true that the number of worms found is small (3 to 8), but on consulting Table VII, it will be seen that equally large doses (6,900 and 23,000) of infective larvae of N. suillus resulted in an almost equally small production (10 to 32) of adult worms. It appears certain

TABLE VII.

Results of Ackert and Payne's experiments at infection of domestic pigs with larvae of N. suillus obtained from naturally infected pigs.

lo. of pig	No. of larvae	Date given	Stage of larvae	How given	Date killed	Result of autopsy	Remarks
1	2500 4400	29.9.21 1.10.21	} ' Infective '	By mouth	Died 5.11.21	32 N. suillus	Pigs 1 and 2 both showed ancylostome ova in their
2	11000 12000	9.10.21 11.10.21	} ' Infective '	Placed on shaven skin	3.12.21	10 adult N. suillus	experiment for which they received anti hel- minthic treatment the results of which are not recorded

therefore that it is extremely difficult to infect the domestic pig with Necators whether the infective larvae used are obtained from other pigs or from the human host.

The present writer while in Amazonas undertook no experiments to infect pigs, owing to the difficulty of obtaining pigs free from Necators and to his inability to distinguish between the pig and the human Necators. One experiment has since been carried out in Liverpool. A pig six weeks old was obtained and kept under observation for seven days; period its faeces were examined daily by the during this saturated salt method (Willis, 1921), but with negative results. On 15.12.22 approximately 400 sheathed larvae obtained from a culture of human faeces were mixed with 2 c.c. of Normal saline and injected subcutaneously in the back of the pig, a similar dose was again given on 29.12.22. The patient from whom these cultures were made was later treated with Carbon tetrachloride and all the 96 worms obtained proved to be N. americanus, it therefore appears reasonably certain that the larvae administered to the pig were those of Necator americanus. Trichuris ova made their appearance in the faeces shortly after the first inoculation and persisted throughout the experiment; no other ova were seen till 19.1.23, when ancylostome-like ova first appeared in the faeces. These ova which were very regular in contour and size, measured 68μ by 37μ , and were quite indistinguishable from those of the human ancylostomes; they were always very scanty in numbers, never more than three being found in a single cover slip preparation made from about two grammes of faeces treated by the saturated salt method. Ancylostome ova were not always found at these examinations, the faeces being sometimes negative for two, or even three days. The last occasion on which ova were found present was 5.2.23, examinations on the subsequent four days being negative. The pig was killed 9.2.23, and in spite of a very careful search of the oesophagus, stomach, intestines, trachea, bronchi, etc., the only helminths found were numerous Trichuris in the caecum. During the last week of the experiment the whole bulk of the faeces was daily examined for any Ancylostomes that might be passed per rectum, this search proved negative, but it is extremely difficult to detect an odd Ancylostome in a large mass of faeces and it is therefore uncertain whether the pig got rid of the worms responsible for the ancylostome-like ova during the four days prior to the autopsy, or whether they were missed at the post-mortem; as the search of the organs was undertaken with great care the former conjecture appears the more likely.

CONCLUSION

No constant differences were found between the Necators of Amazonas pigs and those of man from Amazonas and Jamaica such as would justify the formation of a new species for the pig worm.

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